PB82-167255

Survival and Adult Emergence of Aquatic Insects in Air-Supersaturated Water

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Dec 81

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO.	2.	3.
EPA-600/J-81-524	Journal Article	PB82-167255

4. TITLE AND SUBTITLE	5. REPORT DATE
Survival and Adult Emergence of Aquatic Insects in Air-Supersaturated Water	February 1982
	6. PERFORMING ORGANIZATION CODE 054124
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
A.V. Nebeker, F.D Baker, and S.L Weitz	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Corvallis, Oregon 97333	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED inhouse - journal article
same	14. SPONSORING AGENCY CODE EPA/ORD/600/02

15. SUPPLEMENTARY NOTES

Published in Journal of Freshwater Ecology, Volume 1, Number 3, December 1981

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	unclassified	
	20. SECURITY CLASS *(This page)* unclassified	22. PRICE

EPA Form 2220-1 (9-73)

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NATIONAL TECHNICAL
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EPA-600/J-81-524
Journal Article

Survival and Adult Emergence of Aquatic Insects in Air-Supersaturated Water

A. V. Nebeker, F. D. Baker, and S. L. Weitz

U.S. Environmental Protection Agency
Corvallis Environmental Research Laboratory
Western Fish Toxicology Station
1350 S.E. Goodnight Avenue
Corvallis, Oregon 97333

ABSTRACT

Mayflies Timpanoga hecuba, caddisflies Dicosmoecus gilvipes, mosquitoes Culex peus and midges Cricotopus sp. were tested in the laboratory to determine the effects of air-supersaturated water on survival and adult emergence. The acute 96-hour LC50 value (lethal concentration which killed 50% after 96 hr) for mayflies was 128.9% saturation. Times to death and mean times to 50% death were determined. Adult midges and mosquitoes emerged at 141% and 143%, respectively. Good survival and emergence occurred in controls for all test groups. Adult mayflies did not successfully emerge or survive at concentrations of ≥135%. Adult caddisflies did not emerge at concentrations of ≥134%. Bubbles which were observed in body fluids caused buoyancy problems and raised the insects to the water surface. Data indicate that mosquitoes were highly tolerant and midges were moderately tolerant of air-supersaturated water. Mayflies and caddisflies were relatively sensitive. All insects were more tolerant of air-supersaturated water than fish species.

INTRODUCTION

The purpose of this laboratory study was to determine the effects of air-supersaturated water on the survival and adult emergence of four species of aquatic insects. Many factors are thought to affect the survival of aquatic insects in the field, although it is often difficult to demonstrate a cause and effect relationship. Conditions below large dams are especially perplexing. Water levels fluctuate, flow rates and velocities change, normal daily and seasonal temperatures are radically altered, and normal spring scouring and flushing are often eliminated, creating unnatural sedimentation and increased plant and algal growths. Supersaturated water below dams may also impact insect survival. Those insects which normally inhabit the stream benthos, which are restricted to shallow littoral areas, or which come to the water surface directly prior to adult emergence, may not experience supersaturation effects as do fish.

Because most insects have to be at the water surface for adult emergence it was felt that this critical time should be included in our testing program. The air-breathing mosquito, which is tolerant of low dissolved oxygen levels was tested to determine if it would be more tolerant of supersaturated conditions because of its mode of respiration. Although Nebeker (1976), and Montgomery and Fickeisen (1979) have demonstrated some effects of supersaturated water on aquatic insects, there is little information available relative to the effects of supersaturated water on adult insect emergence.

METHODS AND MATERIALS

The mayfly Timpanoga hecuba pacifica (Allen and Edmunds) (Ephemerellidae), the caddisfly Dicosmoecus gilvipes (Hagen) (Limnephilidae), the midge Cricotopus sp. (Chironomidae), and the mosquito Culex peus Speiser (Culicidae) were the test organisms. All four species were collected locally in western Oregon. Last instar mayfly nymphs and fifth instar (last larval instar) caddisfly larvae were tested. Second or third instar midge and mosquito larvae were tested so that one larval molt could be included along with pupal formation and adult emergence.

Unchlorinated well water with the following average characteristics was used: hardness (as $CaCO_3$) - 23 mg/l, alkalinity (as $CaCO_3$) - 22 mg/l, pH - 7.1. Dissolved oxygen was maintained at or near saturation (controls) or higher, depending upon test concentrations. Chemical analyses were conducted according to the American Public Health Association (1971). Water was supersaturated by injecting compressed air into the water supply under pressure (Nebeker et al., 1976) and then releasing test water to the test facility under normal atmospheric pressure. The percent saturation was controlled by the amount of air injected into the water. Saturation levels were measured with the Weiss Saturometer, the Van Slyke gas analyzer, gas chromatography, and Winkler dissolved oxygen determinations. The formula $BP + \Delta P\text{-}VP/BP \times 100$ = percent saturation; where BP = atmospheric pressure, ΔP = saturometer reading, and VP = water vapor pressure, was used with the Weiss saturometer to calculate total percent saturation values. LC50 values were determined using logistic regression methods (Finney, 1977).

After being supersaturated, the water was delivered to the top of a partitioned ladder-like box, where it cascaded down over the partitions, which serially stripped the water of excess gas, making it possible to draw off any concentration of supersaturated water desired. The water was delivered to 10-liter aquaria for caddisfly testing, small artificial streams for mayfly testing, and 100-liter aquaria for midge and mosquito testing. The midges and mosquitoes were held in stainless steel screen cages suspended in the aquaria. All insects were fed during testing and maintained on natural photoperiod and water temperature ($\sim$ 15 C).

RESULTS

Caddisfly

Last instar (5th) larvae of the caddisfly were collected from the Luckiamute River, Benton County, Oregon (water temperature - 14 C) and transported in moist cloth. Those that appeared active and healthy were placed directly into holding tanks (10-liter aquaria) at 15 C. They were fed Oregon Moist fish food pellets, frozen brine shrimp, and presoaked alder leaves.

The test was started July 12 and terminated November 5. Ten animals were placed in each of ten test aquaria and tested at ten measured concentrations (five nominal concentrations duplicated) plus controls, with concentrations ranging from 134.2% to 149.5%. Maximum water depth was 15 cm; natural photoperiod was used. Daily temperature fluctuations of ~ 0.5-1.5 C occurred around the daily mean, and mean daily temperatures ranged from 15 C in early June to 18.5 C in late August, and to 16 C at the time of adult emergence in October.

Quartz sand and fine gravel were provided for substrate so that the larvae could have case material available at all times. A sheet of fine mesh stainless steel screen was half submersed in the aquaria to serve as an emergence substrate for swimming pupae and adults. The top of each aquarium was covered by a nylon screen cage to retain adults. Immobility of larvae when disturbed, with transfer to control water for recovery as a final check, was used as the criterion for death. The typical caddisfly behavior of hanging out of or leaving the case when stressed or dying was useful in determining death. Time of death could not be determined after the larvae enclosed itself within the pupal case. Emergence of swimming pupae from the case was the next observable stage.

Since the caddisflies did not die immediately, acute 96-hour LC50 data could not be obtained. Bubbles were observed on the larvae within two hours of exposure to supersaturated water, and those at higher concentrations after two weeks exposure appeared more sluggish than those at lower gas concentrations. The animals would anchor themselves to the bottom of the aquaria when not active or feeding. It was useful to shift the animals to the center of the aquaria and then observe them for movement. Those which did not crawl away were checked more carefully.

Once caddisflies closed the pupal case it was not possible to determine time of death, however fungal growths subsequently emerging from and engulfing the stone cases indicated that the larvae or prepupae had died within the case. Table 1 presents mean time to 50% death (TM50) at each concentration. Adults began emerging on October 5 (Figure 1), and the last adult emerged on

October 25. Emergence occurred in the control group (56% = 14 adults from 25 larvae), but not in any of the supersaturated test chambers (134.2% to 149.5%).

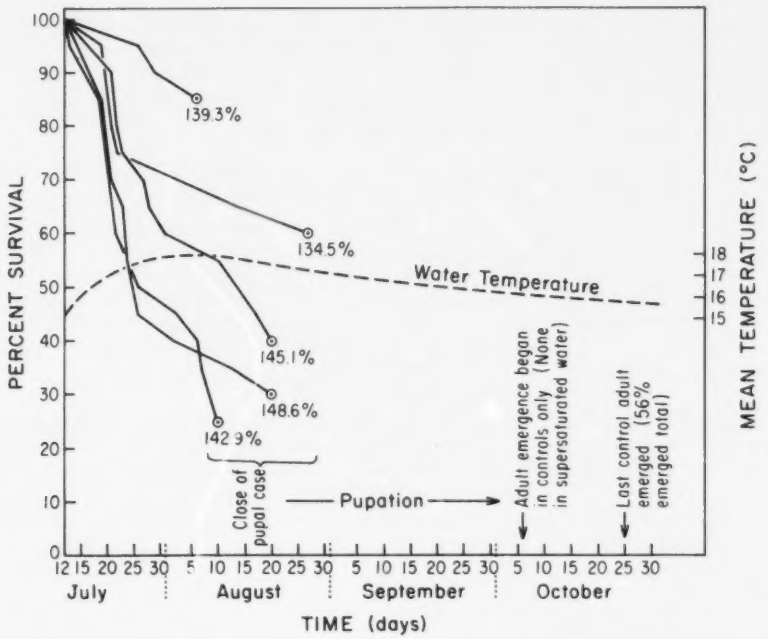

Figure 1. Time table for fifth-instar larval survival, pupation, and adult emergence of the caddisfly Dicosmoecus gilvipes exposed in 10 tanks (5 concentrations) of air-supersaturated water. Data for the duplicate tanks at each concentration are averaged. The 0 represents the time when all larvae had died or closed the pupal case.

Mayfly

Last instar nymphs of the mayfly were collected from the Luckiamute River (water temperature - 17 C) and transported in 20 liters of water containing a cloth substrate. They were held overnight in artificial streams at 16 C. Ten measured concentrations (five nominal concentrations duplicated) plus controls were tested, ranging from 125.0% to 151.2%. Healthy undamaged nymphs were transferred with a dip screen to the ten test streams. Eight insects were placed in each stream, with two streams per nominal supersaturation concentration, resulting in 16 mayflies per

concentration. Fine mesh stainless steel screen was placed partially submersed in each stream as a substrate for nymphs and for adult (subimago) emergence.

Good adult emergence occurred in the two control groups (81% = 13 adults from 16 nymphs; and 62% = 5 adults from 8 nymphs). Seventeen adults attempted to emerge from all supersaturation concentrations except 148.6% and 143.4% during the first 3 days of the test, but most did not complete the emergence process. Successful emergence of 4 adults was completed at and below 135% saturation (Table 2).

Because some of the adults were well developed within the nymphal skin and close to emergence when the test was started, emergence was apparently attempted before the supersaturated water could severely affect their survival or adult emergence. Deaths increased by the second day of exposure and adult emergence did not occur after the third day of the test. Bubbles in the body fluids were visible through the abdominal wall and legs of the mayfly nymphs.

A minimum amount of time was required to develop gas bubble disease and die regardless of the percent saturation exposure (Table 1). This was also apparent from observations of the individual animals which became more sluggish and developed bubbles in their body fluids prior to death. The mean TM50 increased from 1.8 days at 135% saturation to 5.2 days at 126% saturation. The 96-hour LC50 (concentration causing 50% mortality after 96 hr) was 128.9%, with confidence intervals (P = 0.05) of 99.9-135.7%. At 125-126% saturation, mortality was gradual over the 12-day period with one nymph surviving at the end of the test.

Midge and Mosquito

Second and third instar larvae of the midge and the mosquito were collected from fish tanks at this laboratory. They were placed in small cylindrical stainless steel wire cages which were partially submerged in the aquaria, with two cages in each aquarium. The cages had Petri dish bottoms which held food and larval case building materials and provided substrate for the larval tubes. Petri dish covers on the cages retained emerging adults and prevented entrance of stray adults from other areas of the laboratory. Five to ten animals were placed in each cage, depending upon their availability. Food and larval case materials (for midges) consisted of a blended mixture of dead midge larvae and mixed green algae. All animals were transferred with glass pipettes to prevent handling damage. The water temperature for this test was 14 C. Six gas concentrations (117.6% to 141.1%) were used for midge testing, and five concentrations (123.5% to 143.7%) were used for mosquito testing.

Table 1. Mean time to 50% death for caddisflies and mayflies.

Test Animal	Percent Saturation	Mean Time to 50% Death (TM50) (days)
Caddisfly *Dicosmoecus gilvipes*	149.5	10
	147.7	18
	145.7	17
	144.5	28
	142.9	13
	142.9	20
	139.7	>46
	138.8	>46
	134.8	>46
	134.2	45
Mayfly *Timpanoga hecuba*	151.2	1.7
	148.6	1.8
	145.4	1.5
	143.4	1.7
	137.3	1.8
	135.1	1.8
	130.7	2.2
	130.0	2.3
	126.1	5.2
	125.0	2.7

Table 2. Emergence of the mayfly *Timpanoga hecuba* exposed to air-supersaturated water (8 nymphs at each concentration).

Percent Saturation	Nymphs Not Completing Emergence	Nymphs Completing Successful Emergence
151.2	0	0
148.6	3	0
145.4	1	0
143.4	0	0
137.3	2	0
135.1	1	1
130.7	2	2
130.0	1	0
126.1	1	0
125.0	2	1

Adult midges and mosquitoes emerged from all test and control concentrations, indicating that both species are very tolerant of supersaturated water. Forty percent of the midges successfully emerged from the controls; fifty percent emerged from the high concentration (141.1%). Some dead larvae contained air bubbles. Sixty-seven percent of the control mosquitoes emerged successfully; seventy-one percent emerged from the high concentration (143.7%). Some dead larvae also contained bubbles. Similar results occurred in the other gas concentrations for midges and mosquitoes and there was no correlation between gas concentration and mortality.

DISCUSSION

We have shown from this study that aquatic insects are more tolerant to air-supersaturated water than are most fish species (Weitkamp and Katz, 1980). Larval midges and mosquitoes survived, pupated, and emerged as adults from concentrations as high as 143%, a level which kills salmonid fishes in about one hour. Differences also exist between insect species in their susceptibility to high levels of dissolved gases in water. The mayflies are relatively sensitive with a 96-hour LC50 value of 128.9% saturation, and none successfully emerged as adults above 135% saturation. The caddisflies were more tolerant than the mayflies, with 70% of the larvae surviving long enough to close up the pupal case at 148%, though no adults emerged at or above 134%, the lowest concentration tested.

The emergence of mayfly adults was influenced by the fact that incomplete emergence occurred in some instances before the nymphs could be significantly impacted by the supersaturated water. Some of the subimagos were completely developed within the nymphal exoskeleton before being placed in supersaturated water. All but one of the less developed nymphs had died after 12 days exposure to the supersaturated water, with bubbles observable in the body fluids. If mayfly nymphs in natural streams were living at depths below which supersaturation was not affecting them because of increased water pressure, the brief time they spent at the water surface emerging as adults is probably not long enough to inhibit normal emergence. However, those living in shallow water could be significantly impacted by supersaturated water.

The mosquitoes were probably more tolerant since they have air breathing structures which permit significant independence from gas interactions in the water column. The small midge larvae were tolerant of gas levels lethal to mayflies and caddisflies, but no obvious reasons for this were apparent. Their small size, simple respiratory system, and thin exoskeleton which permits more rapid gas exchange, may have precluded internal development of high gas pressures which obviously disrupted normal tissue fluid movements in the larger insect species.

ACKNOWLEDGEMENTS

We wish to thank Dr. N. H. Anderson, Oregon State University, for suggestions on identification and care of caddisflies, R. J. Baker for help collecting the mayflies, J. K. McCrady for assistance with water and gas analyses, and J. D. Andros for statistical assistance.

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